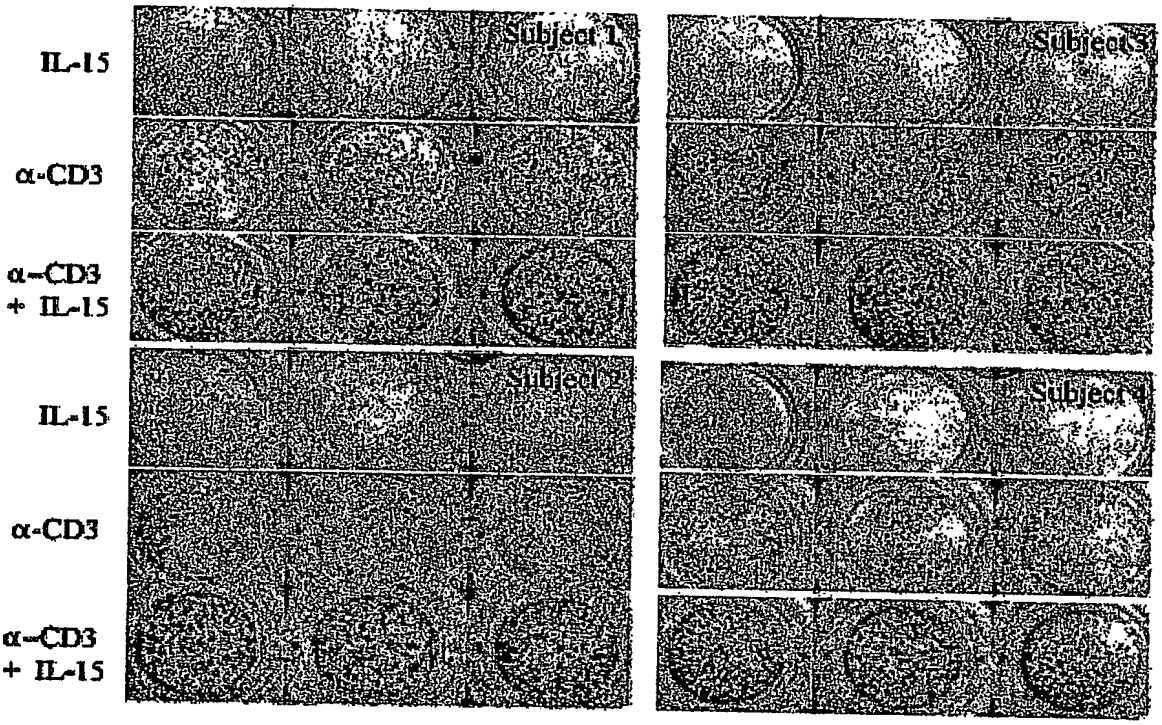


Figure 1

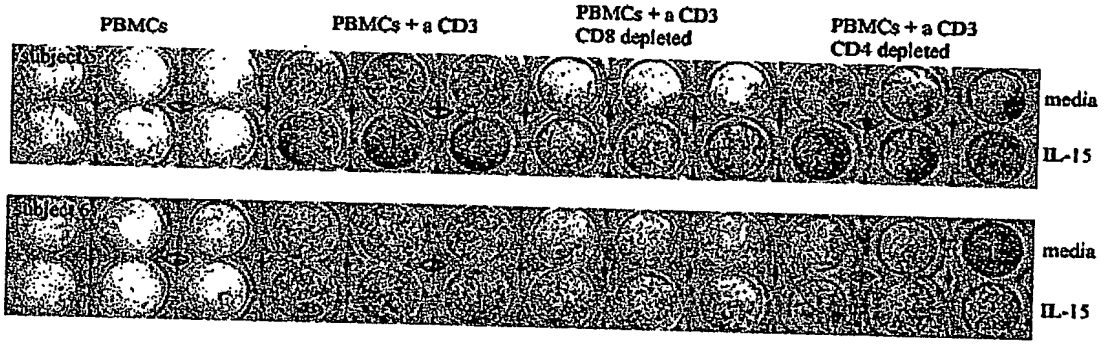

PBMCs
PBMCs + a CD3
PBMCs + a CD3 CD8 depleted
PBMCs + a CD3 CD4 depleted
Subject 5
media
IL-15
Subject 6
media
IL-15

Figure 2

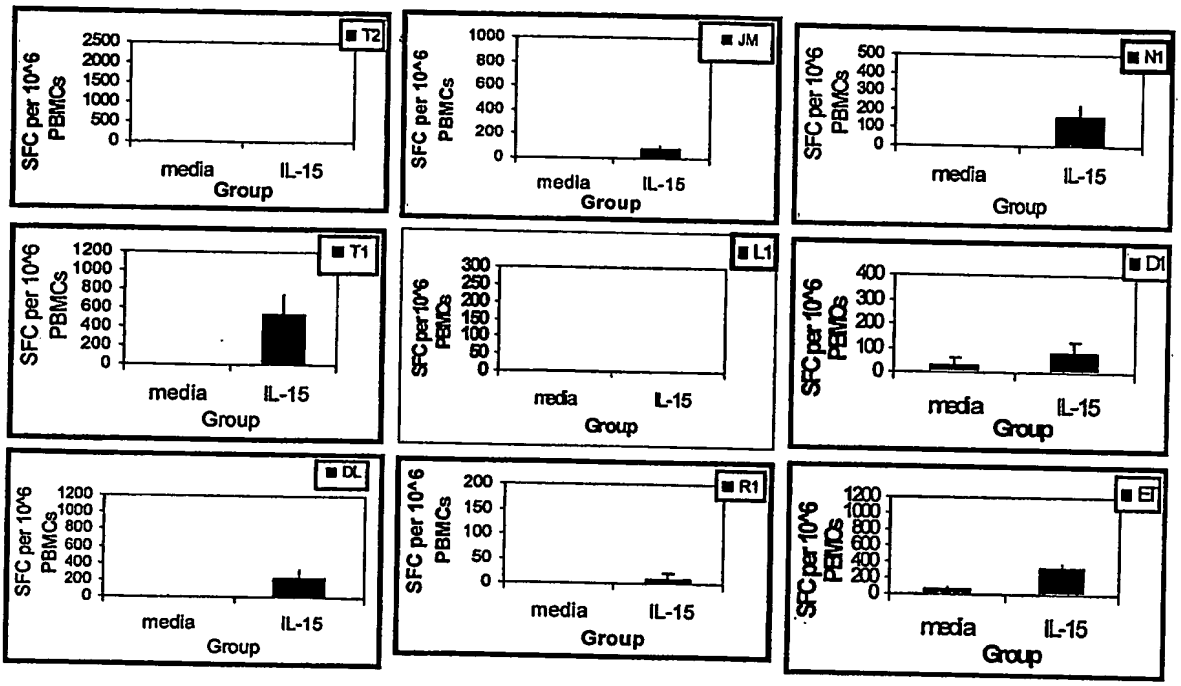


FIGURE 3A

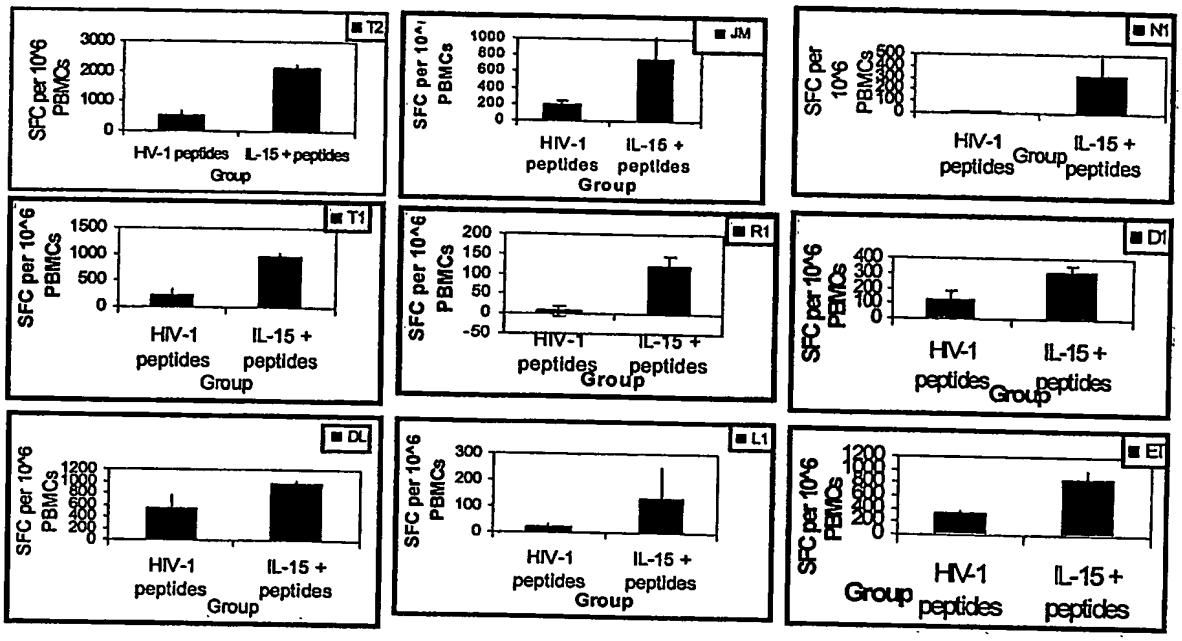

T2
SFC per 10^6 PBMCs
3000
2000
1000
0
HIV-1 peptides
IL-15 + peptides
Group
JM
1000
800
600
400
200
0
N1
T1
1500
1000
500
R1
200
150
100
50
-50
D1
400
300
DL
1200
L1
E1

FIGURE 3B

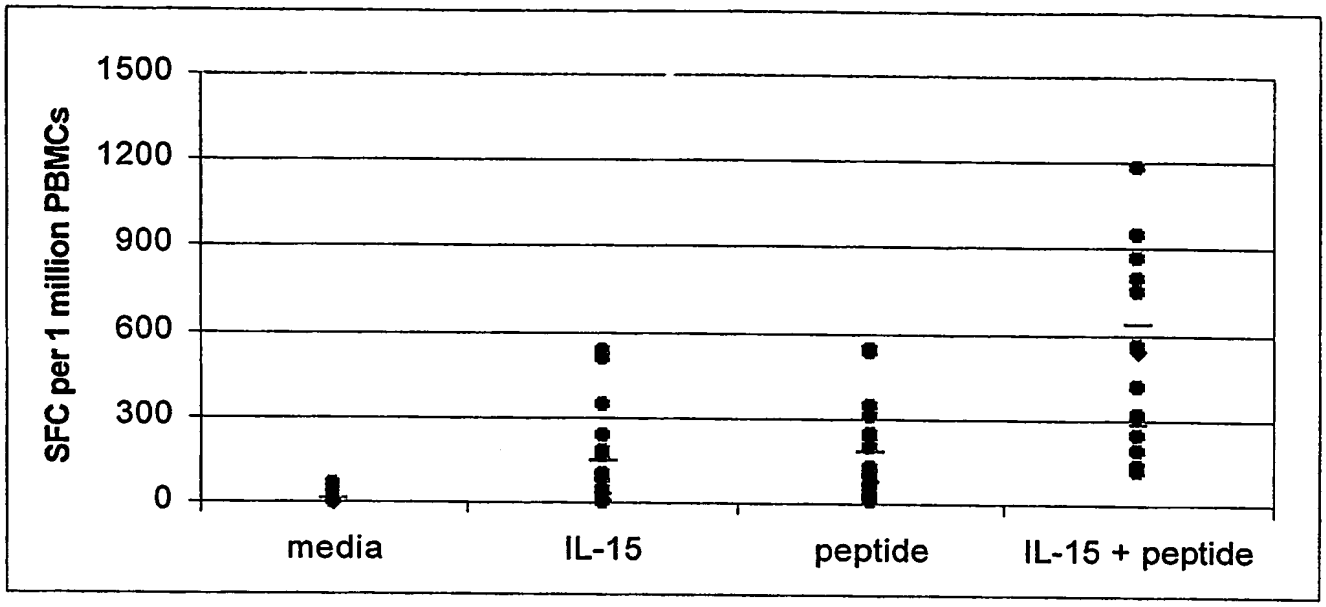


Figure 3C

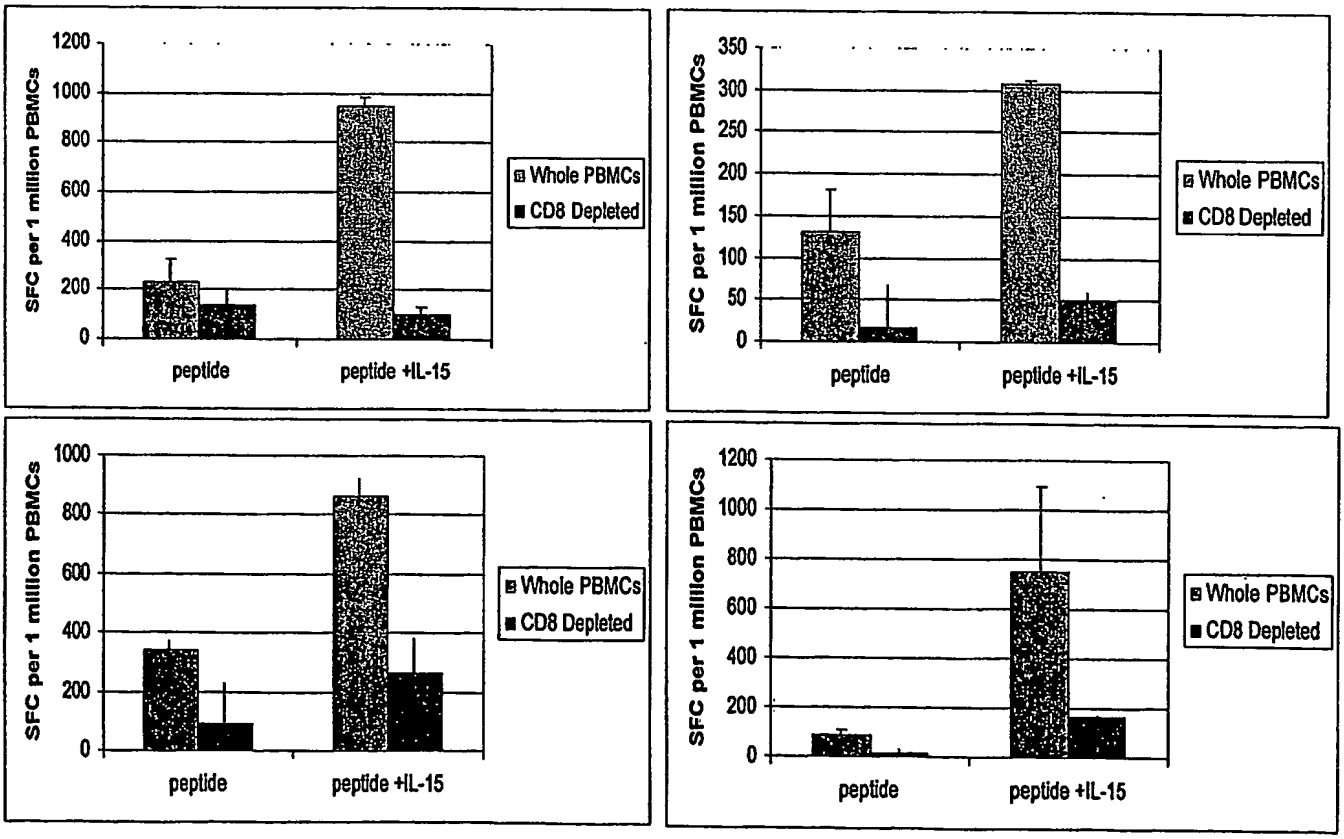
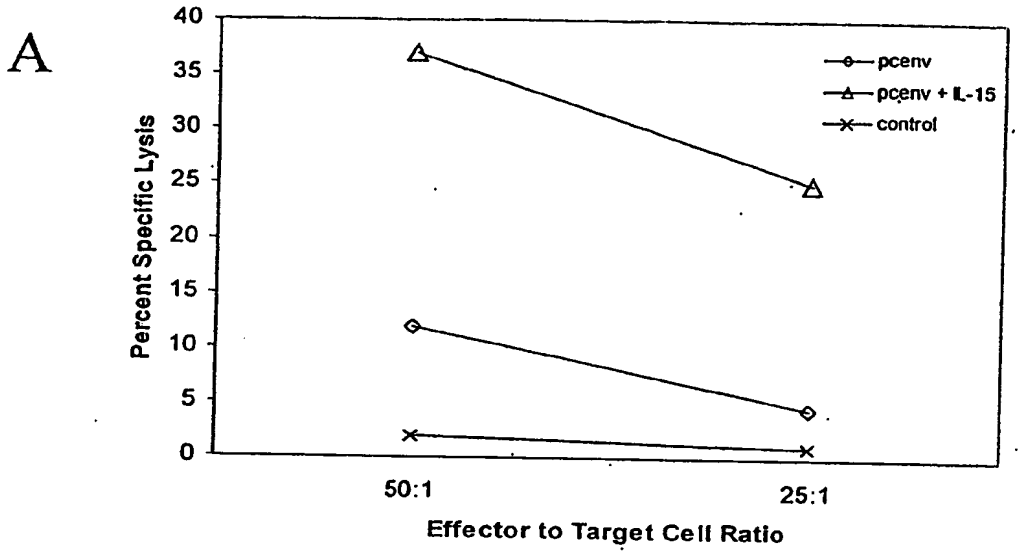
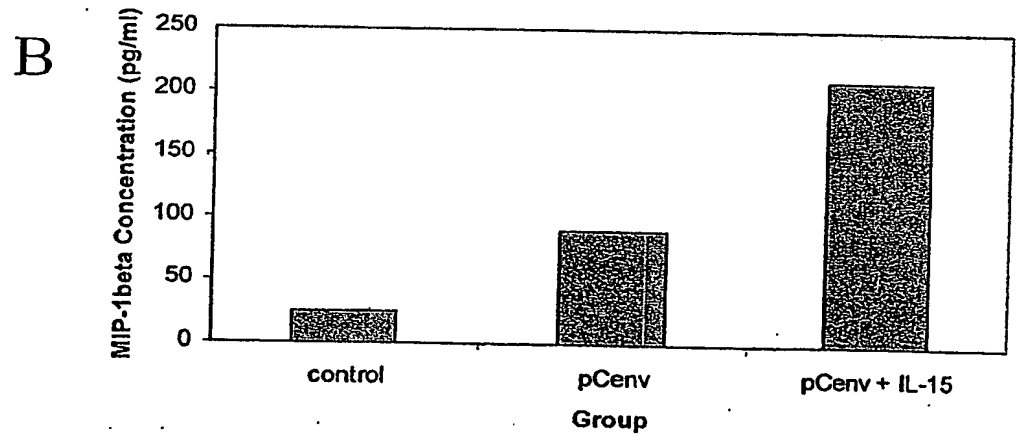


Figure 3D


A
Percent Specific Lysis
40
35
30
25
20
15
10
5
0
pcenv
pcenv + IL-15
control
50:1
25:1
Effector to Target Cell Ratio


B
MIP-1beta Concentration (pg/ml)
250
200
150
100
50
0
control
pCenv
pCenv + IL-15
Group

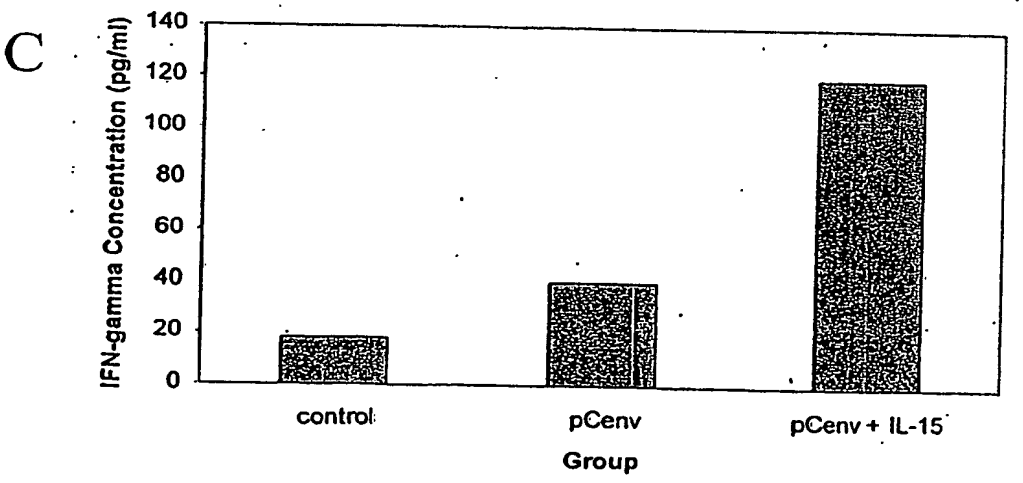

C
IFN-gamma Concentration (pg/ml)
140
120
100
80
60
40
20
0
control
pCenv
pCenv + IL-15
Group

Figure 4

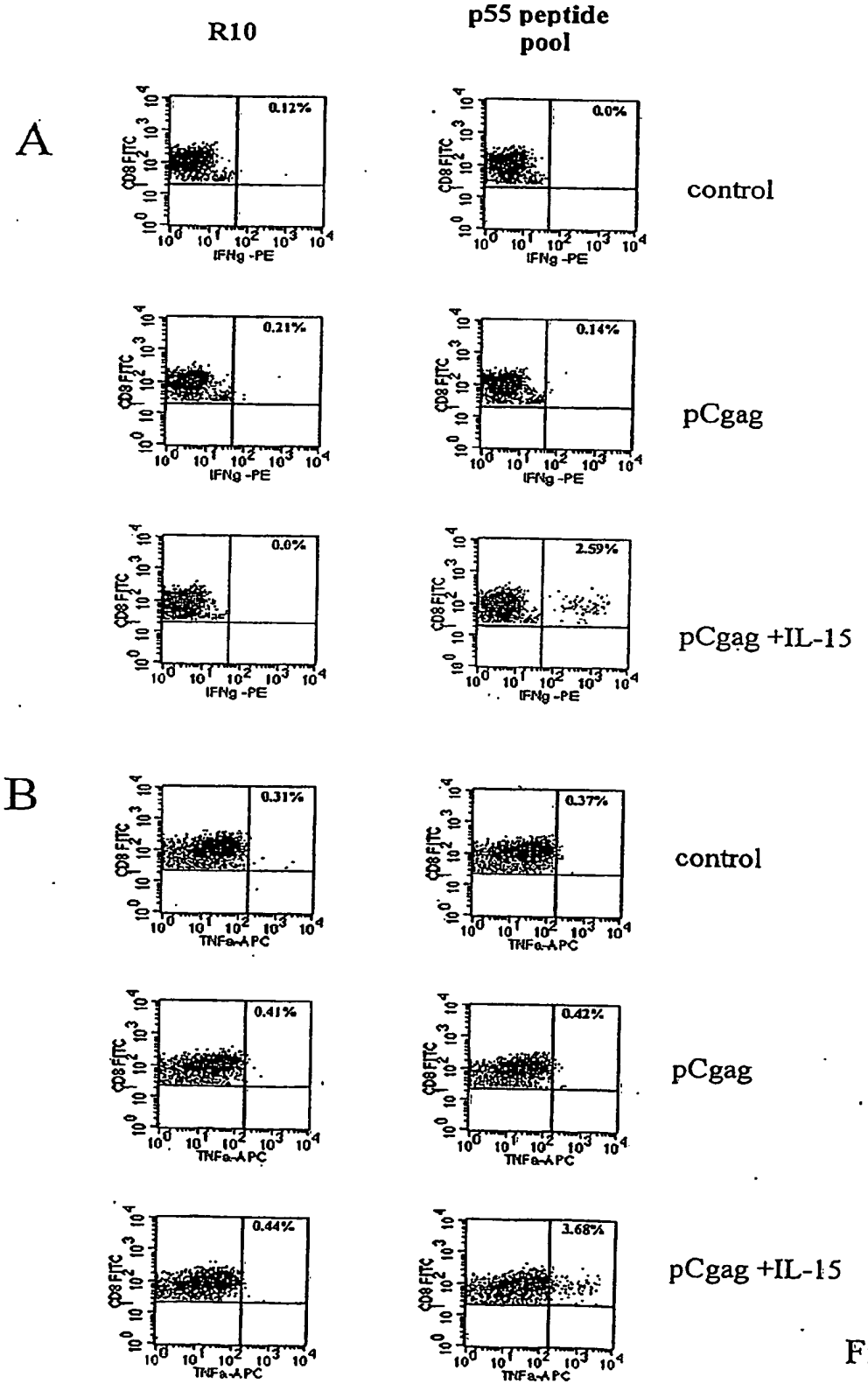

R10
p55 peptide pool
A
0.12%
0.0%
control
0.21%
0.14%
pCgag
0.0%
2.59%
pCgag +IL-15
B
0.31%
0.37%
control
0.41%
0.42%
pCgag
0.44%
3.68%
pCgag +IL-15
CD8 FITC
IFNg -PE
TNFa-APC

Figure 5

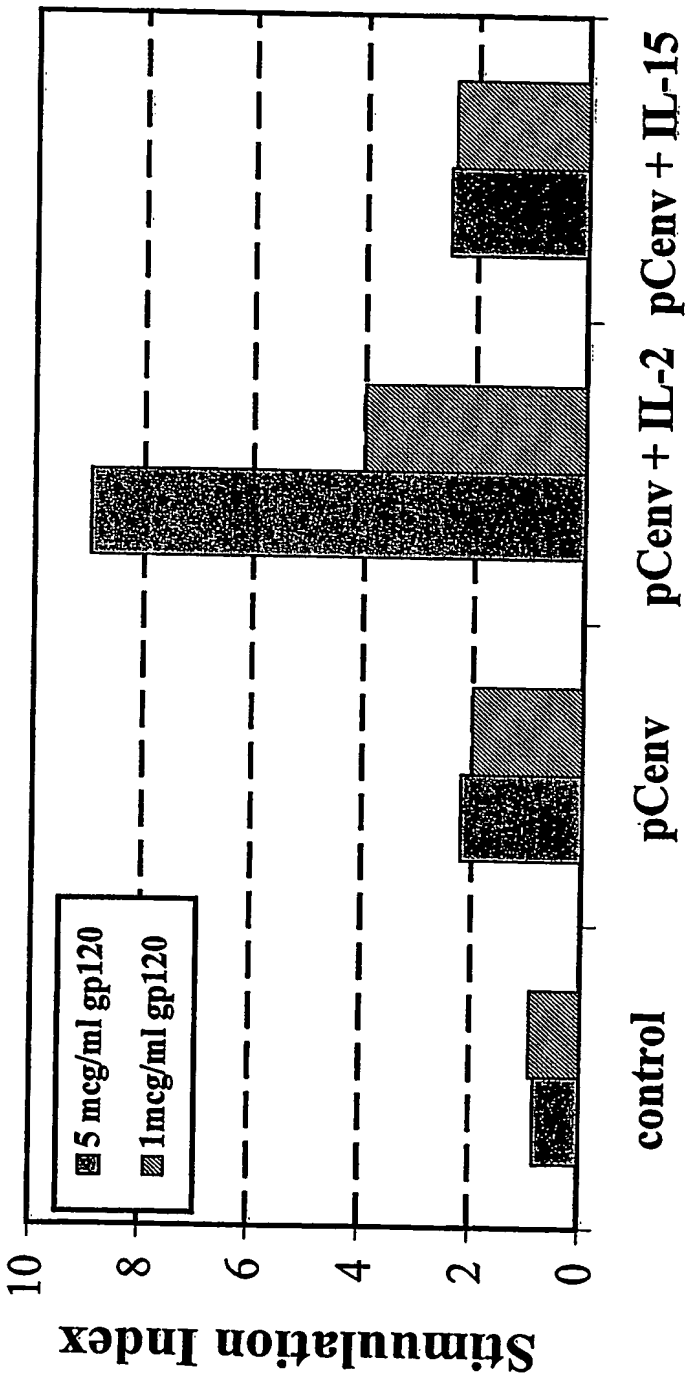

Stimulation Index
10
8
6
4
2
0
5 mcg/ml gp120
1mcg/ml gp120
control
pCenv
pCenv + IL-2
pCenv + IL-15

FIGURE 6

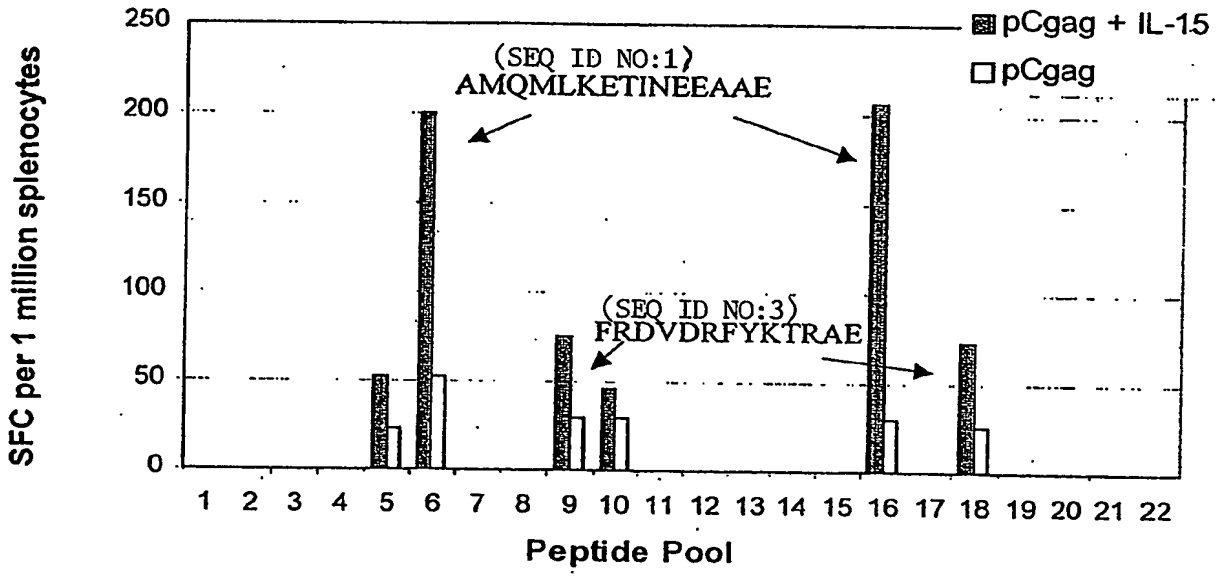
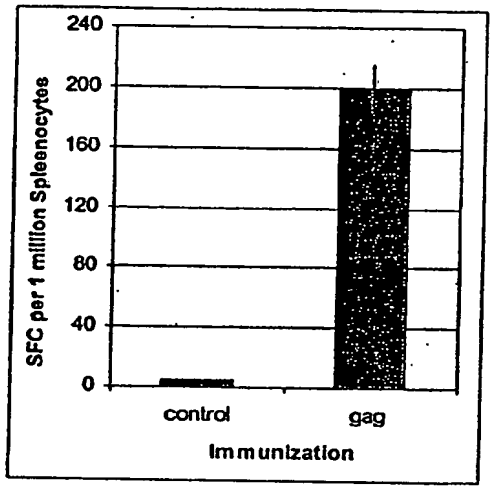
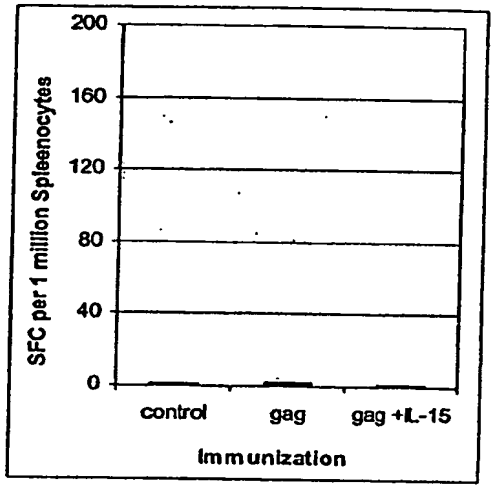

250
200
150
100
50
0
SFC per 1 million splenocytes
pCgag + IL-15
pCgag
(SEQ ID NO:1)
AMQMLKETINEEAAE
(SEQ ID NO:3)
FRDVDRFYKTRAE
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
Peptide Pool

Figure 7

A


240
200
160
120
80
40
0
SFC per 1 million Spleenocytes
control
gag
Immunization

B


200
160
120
80
40
0
SFC per 1 million Spleenocytes
control
gag
gag +IL-15
Immunization

C

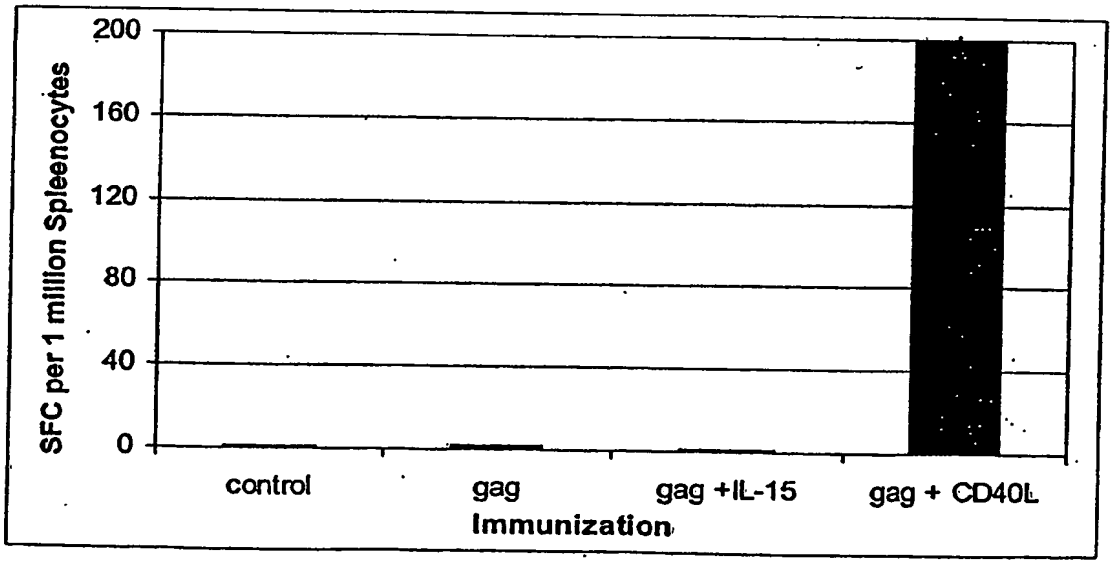

200
160
120
80
40
0
SFC per 1 million Spleenocytes
control
gag
gag +IL-15
gag + CD40L
Immunization

Figure 8

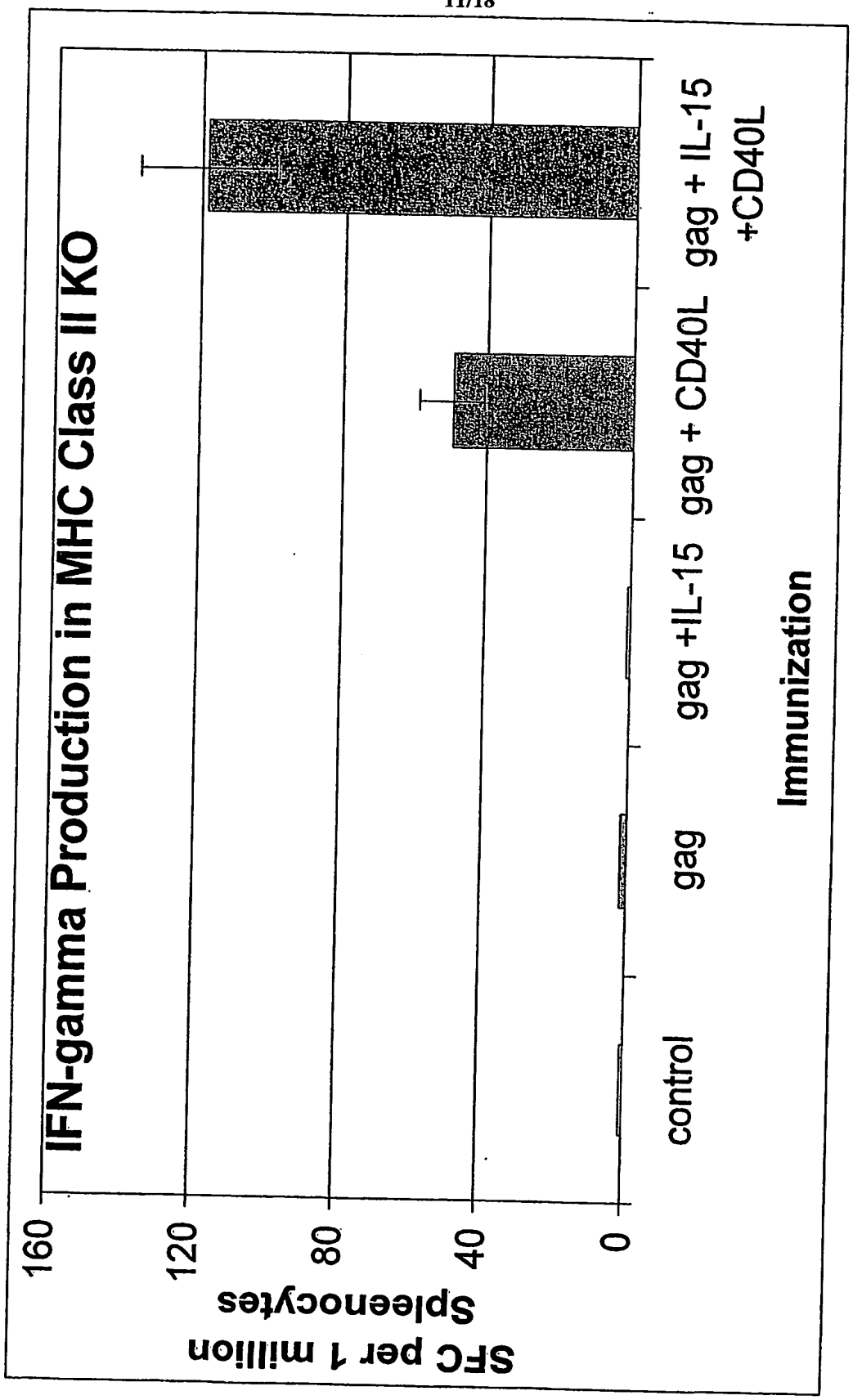

IFN-gamma Production in MHC Class II KO
SFC per 1 million Splenocytes
160
120
80
40
0
control
gag
gag +IL-15
gag + CD40L
gag + IL-15 +CD40L
Immunization

FIGURE 9

Strategy for Increasing Expression of IL-15 through Optimization of IL-15 DNA Constructs for Immunization

- Primers are designed to amplify IL-15 from start of signal peptide, thus upstream inhibitory AUGs are not present in the final IL-15 message.
- Primers are designed to include a strong KOZAK context (GCCGCCACC).
- Removal of the C-terminus negative regulatory element using PCR antisense primer design

Primer Name	*Sense/Antisense*	*Sequence 5' to 3'*
Human IL-15 (LSP)	sense (SEQ ID NO:4)	GCCCCC*GTCGAC* GCCGCCACCATGAGAATTTCGAAACCACATTTGAG
	antisense(SEQ ID NO:5)	ATCGGGC*TCGAG* TCAAGAAGTGTTGATGAACATTTGG
Macaque IL-15 (LSP)	sense (SEQ ID NO:4)	GCCCCC*GTCGAC* GCCGCCACCATGAGAATTTCGAAACCACATTTGAG
	antisense(SEQ ID NO:5)	ATCGGGC*TCGAG* TCAAGAAGTGTTGATGAACATTTGG
Human IL-15 (SSP)	sense (SEQ ID NO:6)	GCCCCC*GGTACC* GCCGCCACCATGGTATTGGGAACCATA
	antisense(SEQ ID NO:7)	ATCGGGG*GATCC* TCAAGAAGTGTTGATGAACAT

Legend: *Restriction Site*, KOZAK, START, STOP CODON

FIGURE 10

Strategy for Increasing Expression of IL-15 through Replacement of 48 amino acid Signal Peptide (LSP) with IgE leader

- Sense primers are designed to start after 48 aa ISP while antisense primer amplifies from stop site.
- Primers are designed to include a strong KOZAK context (GCCGCCACC).
- Sense primer is designed to contain the sequence for IgE leader sequence plus a ATG start site.

Primer Name	Sense/Antisense	Sequence 5' to 3'
Human IL-15-IgE	sense (SEQ ID NO: 8)	GCCCCCG*AATTC* GCCGCCACCATGGATTGGACTTGGATCTTATTTTT
	(SEQ ID NO: 9)	AGTTGCTGCTGCTACTAGAGTTCATTCTAACTGGGTGAATGTAATAAGT
	antisense(SEQ ID NO: 5)	ATCGGG*CTCGAG* TCAAGAAGTGTTGATGAACATTTGG
Macaque IL-15-IgE	sense (SEQ ID NO: 8)	GCCCCCG*AATTC* GCCGCCACCATGGATTGGACTTGGATCTTATTTTT
	(SEQ ID NO: 9)	AGTTGCTGCTGCTACTAGAGTTCATTCTAACTGGGTGAATGTAATAAGT
	antisense(SEQ ID NO: 5)	ATCGGG*CTCGAG* TCAAGAAGTGTTGATGAACATTTGG

Legend: *Restriction Site*, KOZAK, START, STOP CODON

FIGURE 11

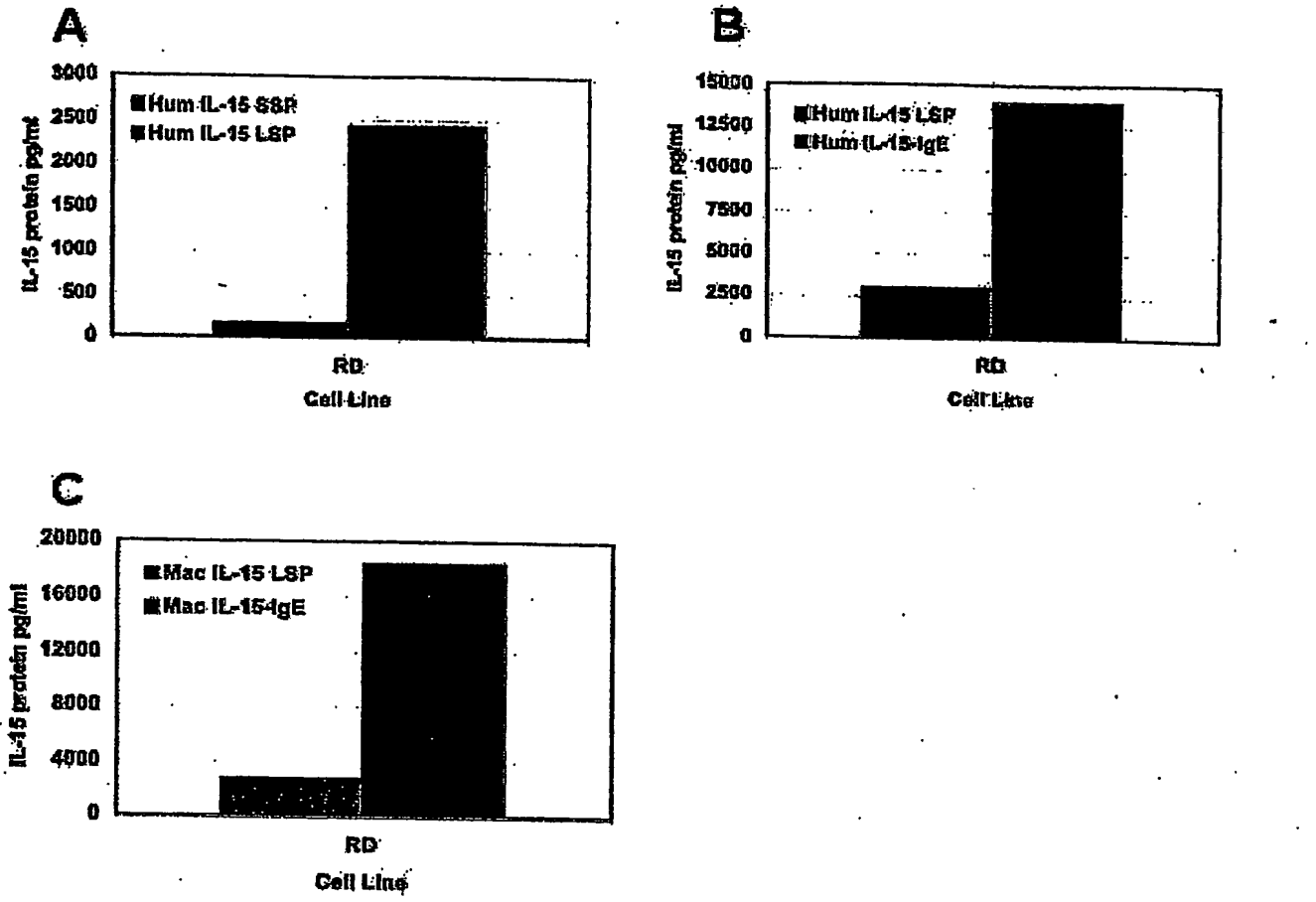

A
3000
2500
2000
1500
1000
500
0
Hum IL-15 SSP
Hum IL-15 LSP
IL-15 protein pg/ml
RD
Cell Line
B
15000
12500
10000
7500
5000
2500
0
Hum IL-15 LSP
Hum IL-15-IgE
IL-15 protein pg/ml
RD
Cell Line
C
20000
16000
12000
8000
4000
0
Mac IL-15 LSP
Mac IL-15IgE
IL-15 protein pg/ml
RD
Cell Line

FIGURE 12

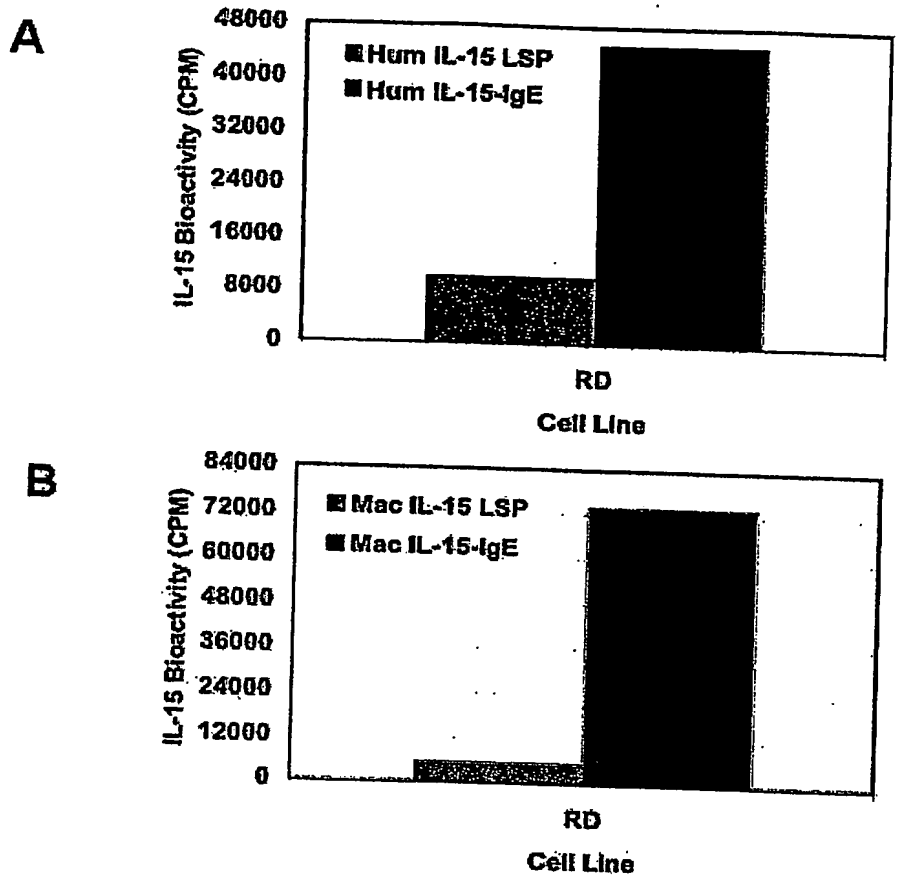

A
48000
40000
32000
24000
16000
8000
0
IL-15 Bioactivity (CPM)
Hum IL-15 LSP
Hum IL-15-IgE
RD
Cell Line
B
84000
72000
60000
48000
36000
24000
12000
0
IL-15 Bioactivity (CPM)
Mac IL-15 LSP
Mac IL-15-IgE
RD
Cell Line

FIGURE 13

Immunization Schedule

Immunization Groups:
Naïve
Vector Control
HIV-1 Gag
HIV-1 Gag/ IL15 constructs

Combinations of 100 µg IL15 Constructs, 50 µg GAG, Each injection, intramuscular

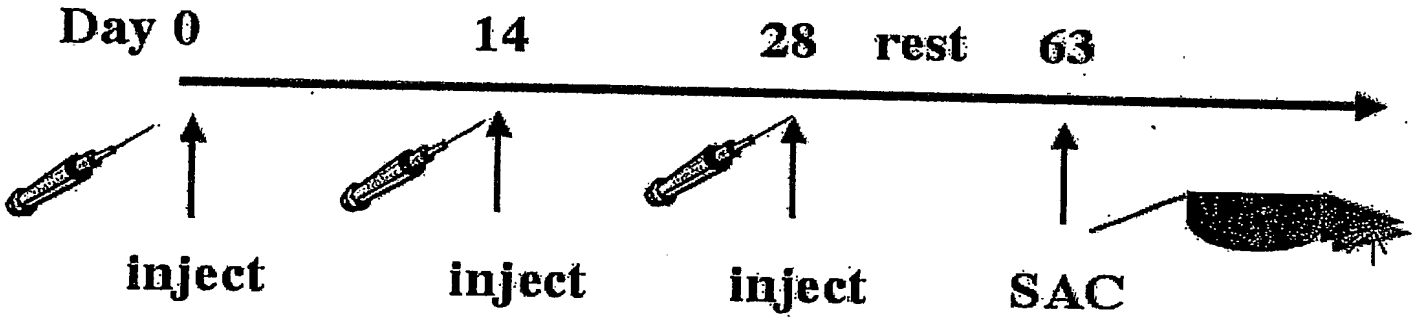


FIGURE 14

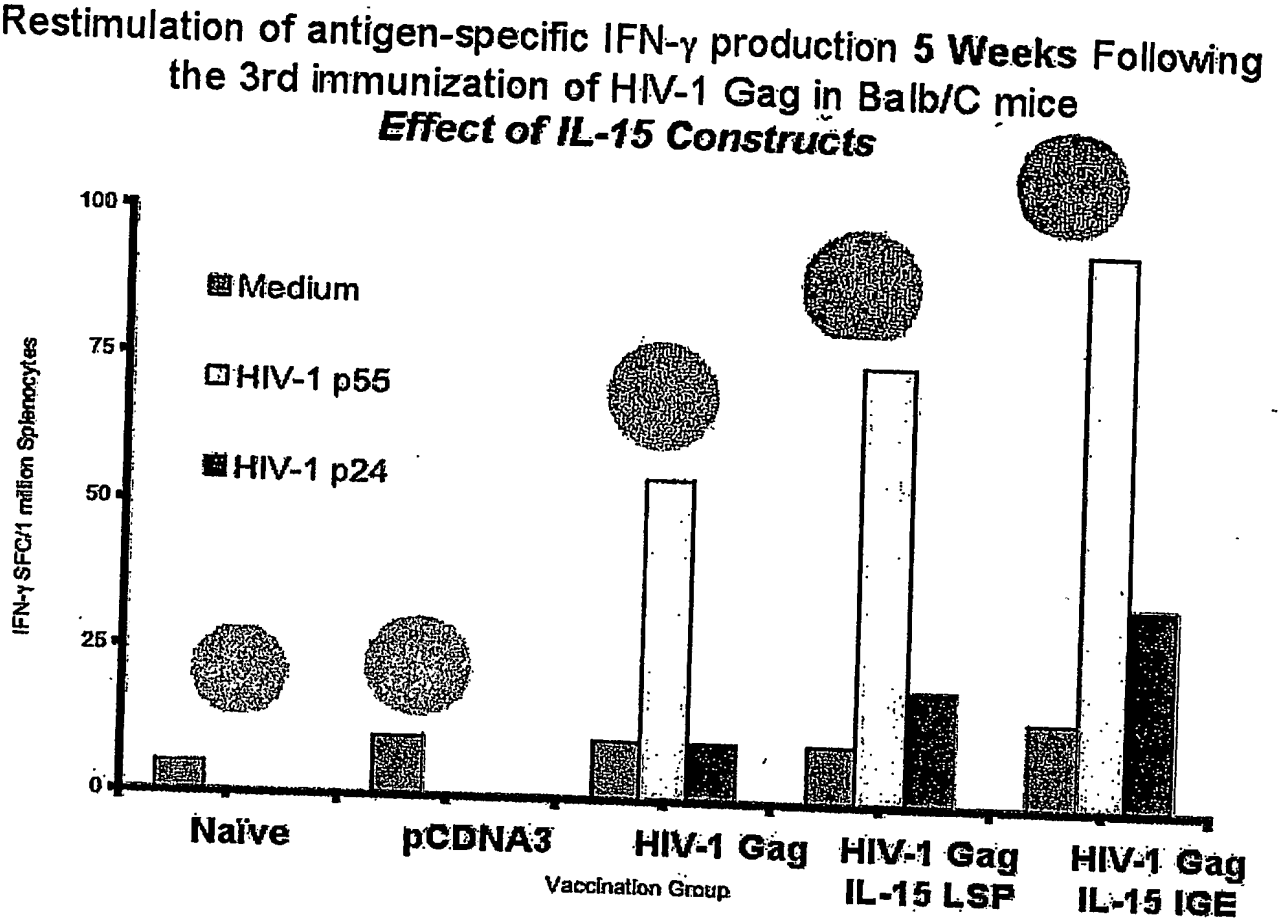
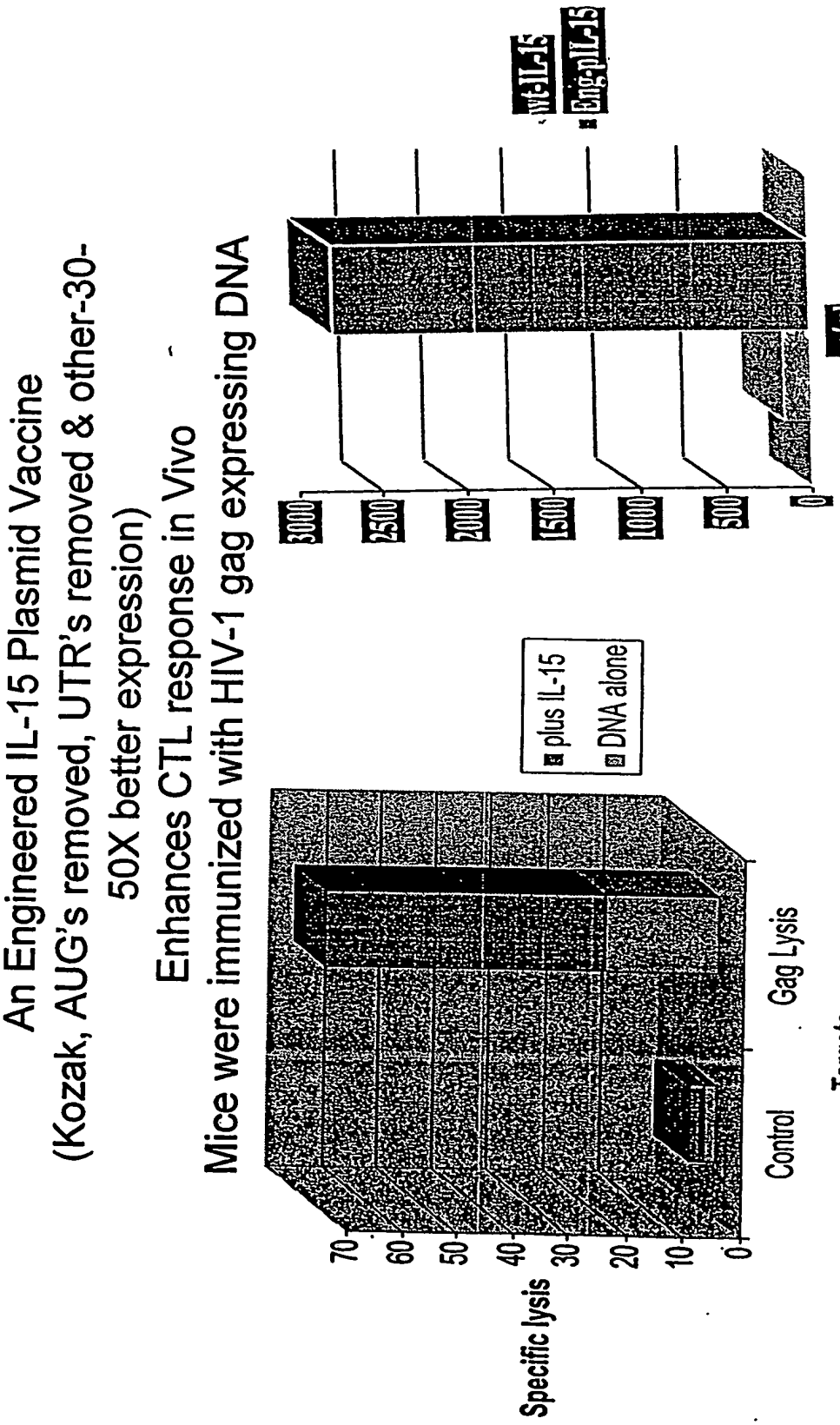

Restimulation of antigen-specific IFN-γ production 5 Weeks Following the 3rd immunization of HIV-1 Gag in Balb/C mice
Effect of IL-15 Constructs
IFN-γ SFC/1 million Splenocytes
100
75
50
25
0
Medium
HIV-1 p55
HIV-1 p24
Naïve
pCDNA3
HIV-1 Gag
HIV-1 Gag IL-15 LSP
HIV-1 Gag IL-15 IGE
Vaccination Group

FIGURE 15



Grabstein et al. (1994) Science 264:965-968, Bamford et al., 1996) PNAS 93:2897-2902
Bamford et al., (1998) J. Immunol 160:4418-4426, Kozak et al., (1991) J. Cell Biol. 115:887-903

FIGURE 16

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